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Review Article

ROLE OF EPOXIDE AND SOLUBLE EPOXIDE HYDROLASE IN CARDIOVASCULAR PHYSIOLOGY

Mohit Gautam, Archana Jain, *Kushwah, M.S., Jyotsana Shakkarpude & Bablu Jamre
Department of Veterinary Physiology, College of Veterinary Science and Animal Husbandry, Mhow (M.P.)-453446
*Corresponding author email: drmoharvet@gmail.com

ABSTRACT
Epoxyeicosatnenoic acid (EET) is arachidonic acid metabolites that importantly contribute to vascular and cardiac physiology. The CYP epoxgenases that convent arachdionic acid to EETs are primanly members of the CYP2C and CYP2J classes (Capdevilla and Falck, 2002) The contribution of EETs to vascular and cardiac function is further influenced by soluble epoxide hydrolase (sEH) that degrades EETs to diols. Vascular actions of EETs include dilation and angiogenesis (Imig, 2012) Myocyte contraction and increased coronary blood flow are the two primary EET actions in the heart. The Presence of the she enzyme in cardiac myocytes regulates the conversion of EETs to DHETs and this regulation impacts on heart functions (Motoki *et al*., 2008) EET vasodilation has also been observed in blood vessels from bovine, canine, rodent and rabbit species (Imig *et al*., 2010) EET and EET anlogs and she inhibitors have an important role in hypertension, cardiac hypertrophy, ischemic injury, and vascular inflammation and atherosclerosis. The development of novel pharmacological and molecular biological tools such as EET agonist and antagonists will be necessary to properly define the cardiovascular action of EET_S, also the angiogenic action in cancer and tumor biology.

KEYWORDS: EET, Cardiovascular, CYP2C, CYP2J and HETEs.

INTRODUCTION

Epoxides /Oxirane are cyclic ether with a three-atom ring, which approximates an equilateral triangle, which makes it highly strained. The strained ring makes epoxides more reactive than other ethers. Highly polarized oxygen carbon bond, basic structure is analogous to that of ethylene oxide. Epoxyeicosatrienoic acids (EETs) are arachidonic acid metabolites that importantly contribute to vascular and cardiac physiology. The contribution of EETs to vascular and cardiac function is further influenced by soluble epoxide hydrolase (sEH) that degrades EETs to diols. Vascular actions of EETs include dilation and angiogenesis (Imig, 2012). EETs also decrease inflammation and platelet aggregation and in general act to maintain vascular homeostasis. Myocyte contraction and increased coronary blood flow are the two primary EET actions in the heart. Epoxyeicosatrienoic acids (EETs)-eicosanoides form by cytochrome p-450 (CYP) pathway from arachidonic acid. Arachidonic acid that resides in sn-2 position of phospholipids in cell membranes. When librated this 20 carbon fatty acid form eicosanoids. The cardiovascular effects of epoxyeicosatrienoic acids (EETs) include vasodilation, antimigratory actions on vascular smooth muscle cells and anti-inflammatory actions. These endogenous lipid mediators are broken down into diols by soluble epoxide hydrolase (sEH), and so inhibiting this enzyme would be expected to enhance the beneficial cardiovascular properties of EETs. sEH inhibitors (sEHIs) that are based on 1,3-disubstituted urea have been rapidly developed, and have been shown to be antihypertensive and anti-inflammatory, and to protect the brain, heart and kidney from damage. Although challenges for the future exist- including improving the drug-like properties of sEHIs and finding better ways to target sEHIs to specific tissues - the recent initiation of the first clinical trials of sEHIs has highlighted the therapeutic potential of these agents.

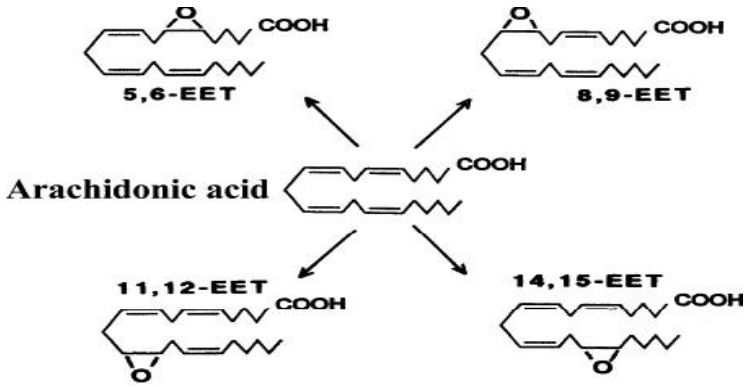
METABOLIC PATHWAY

Arachidonic acid is a major component of cell membranes that resides in the sn-2 position of phospholipids. Once liberated from cell membrane phospholipids, this 20-carbon fatty acid is converted by a series of enzymes to numerous biological active metabolites termed "eicosanoids." Cyclooxygenase (COX) metabolites that include the prostaglandins (PGs) were the first arachidonic acid metabolites to be extensively studied. A major breakthrough that highlighted the potential cardiovascular importance for eicosanoids was the discovery that aspirin inhibited COX enzymes and the formation of PGs. Since that time, drug therapies for cardiovascular diseases, pain, inflammation, and cancer have been developed that manipulate enzymes of the COX metabolic pathway, mimic or antagonize COX metabolites, and inhibit or activate COX metabolite receptors (Hao and Brayer, 2008). Another arachidonic enzymatic pathway is the lipoxygenase (LOX) pathway that is responsible for the generation hydroxyeicosatetraenoic acids (HETEs), lipoxins (LXs), and leukotrienes (LTs). These metabolites have been implicated in pulmonary responses to asthma, inflammation, and atherosclerosis. The third eicosanoid enzymatic pathway is the cytochrome P-450 (CYP)

pathway that contains two distinct enzymatic activities. CYP hydroxylase enzymes generate HETEs, such as 20-HETE, that have cardiovascular and proinflammatory activities (Roman, 2002). Epoxyeicosatrienoic acids (EETs) derived from CYP epoxygenase enzymes also have cardiovascular actions and are anti-inflammatory.

Species	Isoform	EET–regioisomers
Human	CYP2C8	11,12- EET and 14,15- EET
Rat	CYP 2C11	11,12-EET and 14,15-EET
	CYP2C23	8,9-EET, 11,12-EET and 14,15-EET
Murine CYP2J	Cyp2c44	8,9-EET, 11,12-EET and 14,15-EET

The CYP epoxygenases that convert arachdionic acid to EETs are primarily members of the CYP2C and CYP2J classes (Capdevila & Falck, 2002). These CYP epoxygenase enzymes are located in the endoplasmic reticulum and add an epoxide across one of the four double bonds in arachidonic acid to produce four EET regioisomers: 5,6-EET, 8,9-EET, 11,12 –EET and 14,15-EET.



VASCULAR AND CARDIAC LOCALIZATION-CYP EPOX

Human heart microsomes generate 8, 9-EET and 14, 15-EET with high enantioselectivitity for 14, 15(*R*, *S*) - EET. Ventricular myocytes contain the CYP2J3 isoform, and 14, 15-EET is the major EET regioisomer generated by recombinant CYP2J3. The CYP2J2 isoform is also expressed in cardiomyocytes producing 14, 15-EET, 11, 12-EET, and 8, 9-EET. Mouse hearts express the novel Cyp2c50 epoxygenase enzyme but at much lower levels than the abundant liver expression (Theken *et al*., 2011). The presence of the sEH enzyme in cardiac myocytes regulates the conversion of EETs to DHETs, and this regulation impacts on heart functions (Motoki *et al.*, 2008). Epoxygenase enzymes are present in endothelial and vascular smooth muscle cells, and in general, smaller resistance-sized arteries and arterioles have a greater capacity to generate EETs. Human arteries and arterioles express CYP2C8, CYP2C9, CYP2J2, enzymes (Yu Z *et al.*, 2004). Human coronary endothelial cells highly express sEH, and the presence of this enzyme is considerably lower in the vascular smooth muscle cell layers (Enayetallah *et al.*, 2006). Murine and bovine vascular expression has determined that the Cyp2C isoforms are primarily responsible for EET generation. Rat endothelial cells express CYP2C11 and CYP2C23 epoxygenase enzymes. Renal microvessels have a higher CYP2C23 expression, whereas CYP2C11 predominates in others such as the mesenteric and coronary resistance arteries. In mice, the predominant vascular epoxygenase enzyme is the Cyp2c44

Circulating blood cells could be a significant source for and have the potential to regulate EET levels. EETs are esterified in phospholipids in erythrocytes isolated from a number of species including humans (Jiang *et al.*, 2007). Epoxidation of arachidonic acid is catalyzed by hemoglobin in red blood cells (Jiang *et al.*, 2007). Erythrocyte EETs can serve as a reservoir for release t hese and to vasodilate, inhibit platelet aggregation, and decrease inflammation. Red blood cells also have sEH activity that hydrolyzes cis-EETs and trans-EETs. Human peritoneal macrophages are another circulating cell that has the capacity to generate EETs and their corresponding DHETs.

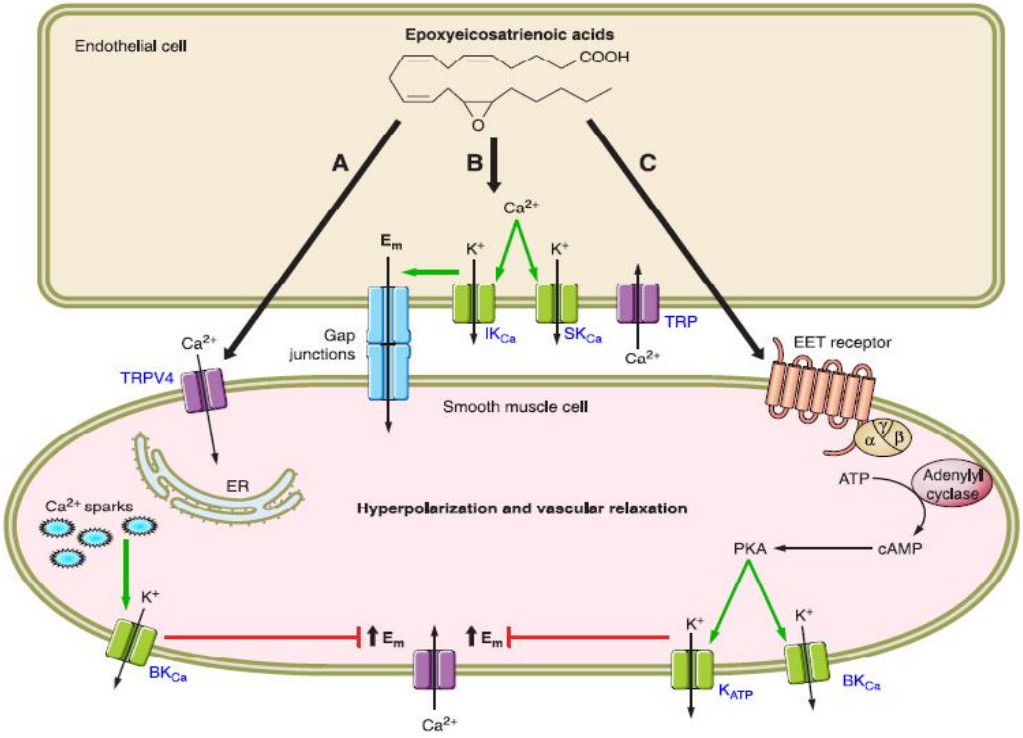
MECHANISM

EETS Vesodilatoin mechanism is mediated by regiooisomeric EET in various organs.

A; EETS grnerated by endothelial cell activate TRPV4 channels on vascular smooth muscle cell, calcium influx through TRPV4 channels causes Ca sparks from the endoplasmic reticulam. Ca sparks activated large – conduction Ca –activated K channels resulting in K efflux from tha smooth muscle cell and membrane hyperpolarization.

B; EETS activate endothelial cell transient receptor potential (TRP) channels resultimg in Ca influx. An increase in endothelial cell Ca activates small-conductance (Skca) and intermediate-conductance (Ikca) K channels to cause membrane hyperpolarization.Endothelial membrane hyperpolarization spreads to the vascular smooth muscle cell via gap junctions.

C; EETS released by endothelial cell activate an unidentified receptor stimulating Camp production via activation of adenylyl cyclase by guanine nucleotide protein. Subsequent protine kinase A (PKA) activation by Camp resulting in activation of Bkca and K ATP ,K efflux and smooth muscle cell hyperpolarization.



FUNCTION OF EET

Vasodilation

EET vasodilation has also been observed in blood vessels from bovine, canine, rodent, and rabbit species (Imig *et al.*, 2010). EETs could have actions on both endothelial and vascular smooth muscle cells. EETs were vasodilators and led to speculation that they could possibly serve as an endothelium-derived hyperpolarizing factor (EDHF). Endothelium-dependent vasodilation to bradykinin and acetylcholine in large blood vessels such as the aorta were largely nitric oxide mediated, resistance-sized blood vessels demonstrated a nitric oxideand COX-independent vasodilation. The most important function of EET is vasodilation especially in small resistance vessels. 14, 15-EET has been observed to produce relaxation of isolated coronary microvessels at low concentrations (Fang *et al* 2001). Recent observations in coronary preparations found that the EDHF response in bovine coronary arteries is inhibited by the EET antagonist 14,15-epoxyeicosa-5(*Z*)-enoic acid. And also EET is the transferable mediator of vasorelaxation in a perfused system consisting of donor and detector coronary arteries (Gauthier *et al.*, 2005). EDHF mechanisms that do not involve EETs but includs the release of lipoxygenase products or hydrogen peroxide from the endothelium.

Species	Major vessel	EEES	Action
Rat	Renal	11,12-EET, 14,15-EET	Vasodialation
Rat	Cerebral	8,9-EET, 11,12-EET	Vasodialation
	Intestinal	11,12-EET, 14,15-EET	Vasodilation
Human	mammary	11,12-EET	Vasodialation
Rabbit	Pulmonary	5,6-EET, 14,15-EET	vasodialation
Piglet dog		5,6-EET	

Ion Channel Activation by EETs

Studies in the bovine coronary artery with 11, 12-EET indicates that activation of the BK_{Ca} channel is mediated by $G\alpha_s$ protein in a process that involves ADP-ribosylation. This process occurs in platelets, decreasing platelet adhesion to the endothelium and in airway smooth muscle, producing bronchodilation through hyperpolarization. Inhibition of smooth muscle Cl^- channels also is involved in the mechanism through which EETs produce relaxation of the airway smooth muscle. EETs are reported to affect other ion channels, including the K_{ATP}, Na^+, and L-type Ca^{2+}channels. EETs bind to the myocardial K_{ATP} channel and thereby reduce its sensitivity to ATP by an allosteric alteration of the ATP binding site. Activation of the mitochondrial K_{ATP} channels protects the myocardium against ischemia-reperfusion injury, suggesting that myocardial preconditioning may occur through a direct interaction

between EETs and the channel. However, activation of the p42/p44 MAPK pathway also appears to be involved in myocardial preconditioning, and studies in mice with targeted deletion of the sEH support a mechanism involving EET-mediated activation of the PI3K signaling pathways and K^+ channels. In addition to activating the myocardial K_{ATP} channel, EETs inhibit the myocardial Na^+ channel by decreasing the probability of channel opening.

Anti-inflammatory Effects of EETs

EETs produce an anti-inflammatory effect on the endothelium by inhibiting cytokine-induced NF-κB transcription (Node *et al.,* 1999**)**. 11, 12-EET produces the most potent effect in bovine aortic endothelial cells. It inhibits IKK-mediated phosphorylation of IκBα, maintaining NF-κB in an inactive state (Spiecker and Liao, 2005). 11, 12-EET also enhances fibrinolysis by activating tPA gene expression through a cAMP-driven promoter. This involves a $G\alpha_s$ protein-mediated signal transduction mechanism. Likewise, a cAMP-PKA signaling pathway mediates the inhibitory effect of 11, 12-EET on rat aortic smooth muscle cell migration.

Angiogenesis

There is increasing evidence that EETs stimulate angiogenesis (Fleming and Busse, 2006). However, the signaling pathway that mediates this process appears to differ depending on the species, type of endothelium, and the EET regioisomer that initiates the process. However, other studies with 11, 12-EET has indicated that the angiogenic process is initiate phosphorylation and inactivation of the forkhead transcription factors FOXO1 and FOXO3a, which decreases the cyclin-dependent kinase inhibitor. This pathway is activated by phosphorylation of the epidermal growth factor (EGF) receptor. Still another angiogenic signal transduction pathway has been reported for human umbilical vein endothelial cells, this one involving cAMP-PKA activation, COX-2 induction, and PGI_2 synthesis.

Cardiac Function

EETs increase coronary blood flow and improve cardiac function when investigated under normal physiological conditions. EETs do act as EDHFs through activation of vascular smooth muscle cell KCa channel in the coronary microcirculation (Larsen *et al.,* 2006). Epoxygenase metabolites of docosahexaenoate also dilate coronary arterioles by activating KCa channel.

In addition to actions on the coronary circulation, epoxygenase metabolites also influence cardiac myocytes . They utilize different cell membrane channels and signaling mechanisms to impact cardiac myocyte function. EETs have complex actions on cardiac cells that could involve multiple cell membrane channels. Subsequent studies have provided evidence that EETs acting on cardiac myocyte Na+ channels, L-type Ca2+ channels, and KATP channels are potential mechanisms by which EETs can alter heart contractility (Lu *et al* ., 2006).

Cardiac Na^+ channels are necessary for action potentials in cardiac myocytes. 8, 9-EET has been demonstrated to modulate Na^+ channel gating behavior to act as a voltage-dependent inhibitor of cardiac Na_ channels. Other EETs also inhibited the Na^+ current in cardiac cells, whereas 8,9-DHET had a small effect on cardiac Na^+ (Lee *et al.,* 1999). Cardiac specific overexpression of the epoxygenase enzyme CYP2J2 shortened cardiac myocyte action potentials most likely due to enhanced maximal peak transient outward K^+ currents (Nithipatikom *et al.,* 2010). EETs act via cAMP/PKA-dependent phosphorylation of the L-type Ca2_ channel to regulate heart contraction.

EET INTERACTIONS WITH HORMONAL AND PARACRINE FACTORS

EETs also influence vascular responses to mechanical stimuli as well as hormonal and paracrine constrictor and dilator factors. Numerous studies have demonstrated a contribution for EETs to acetycholine and bradykinin-elicited EDHF-mediated vasodilation. CYP epoxygenase metabolites account entirely for skeletal muscle arteriolar dilations to acetylcholine. EETs are also an important component to the conducted dilation in response to acetylcholine in arterioles. In this case, the conduction of the hyperpolarization along the arteriole is dependent on CYP-derived epoxygenase metabolites. There is also a contribution of EETs to shear stress-dependent hyperpolarization and dilation of skeletal muscle arterioles. Thimerosol is another endothelium-dependent vasodilator that has a nitric oxide- and PG-independent dilatory component. EETs appear to mediate the EDHF portion of thimerosol renal vasodilation. EETs have also been demonstrated to contribute to vasodilation in response to adenosine activation of the adenosine A_{2A} receptor (Nayeem *et al.,* 2010). Adenosine increases EET generation in blood vessels, and epoxygenase inhibition greatly attenuates the vasodilation to adenosine or A_{2A}receptor agonists. Activation of transcriptional factor AP-1 in endothelial cell activate promoter region on *Ephx2 and* increased sEH protein expression.

EETs have the capacity to increase their contribution to decrease vascular resistance when endothelial nitric oxide levels are decreased. There is an inhibitory interaction between nitric oxide and EETs such that nitric oxide inhibits endothelial epoxygenase generation of EETs. Likewise, a product of the nitric oxide metabolic pathway, hydrogen peroxide (H_2O_2), has been demonstrated to inhibit EET production by human recombinant CYP2C9 and CYP2J2 epoxygenase enzymes (Larsen *et al.,* 2008). Redox control of CYP epoxygenase by H_2O_2 modulate vascular availability of EETs. Increased afferent arteriolar reactivity to angiotensin II in hypertension is blunted by 11,12-EET or sEH inhibition (Zhao *et al.,* 2004). Angiotensin II upregulation of vascular sEH appears to be at the transcriptional level. Angiotensin II activation of the transcriptional factor AP-1 in endothelial cells that subsequently activates the promoter region on *Ephx2* resulted in an increased sEH protein expression. An interaction with endothelin-1 and endothelial generation of EETs can influence vascular tone. Endotheline-1 result in endothelial generation of EETs that appose vasoconstriction (Iming *et al.,* 2000). The epoxygenase inhibitor MS-PPOH enhanced endothelin-1 arteriolar constrictor responses but did not alter endothelin-1 calcium responses in freshly isolated vascular smooth muscle cells. EETs can oppose vasoconstrictor stimuli to maintain proper vascular tone. endothelin-1 vasoconstrictor responses are attenuated in mice that have

transgenic CYP2C8 or CYP2J2 expression in endothelial cells (Lee e*t al.,* 2010). Myogenic constriction in response to increases in perfusion pressure enhanced in presence of CYP epoxygenase inhibitor. Experimental evidence has provided convincing evidence that endothelial-derived EETs can influence responses to mechanical, hormonal, and paracrine stimuli with regard to vascular tone. EETs in general contribute to endothelial-dependent dilator responses and oppose vasoconstrictor responses. The fact the EETs can modulate hormonal responses and that many hormones such as angiotensin II can influence vascular proliferation as well as vascular tone suggested that EETs could also modulate vascular growth processes.

USE OF EETs AND sEH INHIBITOR

The importance of EETs and sEH in cardiovascular disease has been driven by experimental findings demonstrating that the epoxygenase pathway is a key component in the regulation of vascular and cardiac function that can be altered in disease states (Iming *et al.*, 2009). Genetic based evidence in humans has also found that the epoxygenase pathway could importantly contribute to cardiovascular diseases. *EPHX2* genetic polymorphisms that result in amino acid substitutions can influence sEH activity (Enayetallah *et al*., 2006).

These genetic polymorphisms have been linked to cardiovascular disease incidence. The Atherosclerosis Risk in Communities (ARIC) study found the *EPHX2* K55R polymorphism was associated with an increase in the risk for the incidence of coronary artery disease. Likewise, the Coronary Artery Risk Development in young adults (CARDIA) study determined that African Americans having one allele that results in *EPHX2*R287Q substitution increased the risk for coronary artery calcification. Ischemic stroke and hypercholesterolemia are two other cardiovascular diseases that are associated with genetic variation in *EPHX2*. More recently, genetic variations in *EPHX2* were linked to vasodilator responses in humans. Intriguingly, genetic variation in *EPHX2* has been associated with heart failure and vascular disease risk in rat models of cardiovascular disease. Thus there is increasing evidence that genetic variation in the epoxygenase enzymatic pathways is associated with cardiovascular diseases and can impact on cardiovascular function. EET and EET analogs and sEH inhibitors is that the epoxygenase pathway has an important role in hypertension, cardiac hypertrophy, ischemic injury, and vascular inflammation and atherosclerosis. The possible contribution of EETs to blood pressure control and hypertension was first noted in studies where rats treated with an epoxygenase enzyme inhibitor and administered high salt became hypertensive. A number of subsequent studies determined that increased renal epoxygenase enzymes and EET generation in response to a high-salt diet was required for the proper vascular and renal tubular function to maintain cardiovascular homeostasis (Sporkova *et al.,* 2011). Further experimental studies determined that renal vascular sEH expression was increased in angiotensin-dependent hypertension. Administration of sEH inhibitors to increase EETs lowered blood pressure in a number of experimental animal models of hypertension (Ghosh *et al.,* 2008). Although sEH inhibition lowered blood pressure, it did not return values down to normal. Kidney, vascular, heart, and brain damage associated with hypertension is reduced with sEH inhibition or EET analog administration. Hypertension-induced vascular hypertrophy was decreased and afferent arteriolar constriction to angiotensin normalized by sEH inhibition. Decreased renal glomerular injury was observed when sEH inhibitors were administered prior to or after the establishment of hypertension (Manhiani *et al.,* 2009). Angiotensin-induced hypertensi on in Goto-Kakizaki rats did not have a decrease in blood pressure in response to sEH inhibitor treatment but did have decreased renal vascular and glomerular injury, and inflammation (Olearczyk *et al.,* 2009). Decrease renal macrophage infiltration during hypertension.

Cardiac hypertrophy associated with DOCA-salt hypertension is also decreased by sEH inhibitor administration. Likewise, cerebral ischemic injury in hypertensive rats is greatly reduced by sEH inhibition. More recent studies have used newly developed EET analogs and determined their beneficial actions in cardiovascular diseases. An 11, 12-EET analog, NUDSA, has been evaluated in two cardiovascular studies (Imig *et al*., 2010). NUDSA dilated afferent arterioles to a similar extent as 11, 12-EET and lowered blood pressure in SHR and angiotensin-dependent hypertension. Administration of NUDSA reversed the metabolic syndrome phenotype in heme-oxygenase-2 deficient mice. NUDSA treatment for 2 wk decreased body weight, improved glucose regulation, and improved endothelial-dependent relaxation. Interestingly, renal Cyp2c44 expression was increased by NUDSA administration, suggesting that induction of the epoxygenase enzyme could contribute to the biological actions of this EET analog. These studies have provided significant evidence that EET analogs and sEH inhibitors have the ability to improve vascular function and protect organs from hypertensive injury through actions on multiple cell types. Cardioprotection is another area where therapeutic targeting of the epoxygenase pathway has demonstrated promise. These experimental studies have focused on cardiac hypertrophy and ischemia reperfusion injury (Imig *et al.,* 2010). The beneficial effects of EETs and sEH inhibition to prevent the development of left ventricular hypertrophy have been assessed in various animal models. Subsequent studies evaluated sEH inhibition in mice with pressure overload. Mice were treated with sEH inhibitors prior to or following the establishment of left ventricular hypertrophy. Decreased cardiac hypertrophy was associated with decreased NFκB activation in cardiac myocytes in mice administered sEH inhibitors. Interestingly, sEH inhibition also had an antiarrhythmic effect in pressure overload mice.

Improved cardiac function following ischemia reperfusion injury has also been observed with EET and EET analogs, cardiac myocyte overexpression of CYP epoxygenase enzymes, sEH inhibition, and *Ephx2* gene-deficient mice . EETs administered to guinea pig hearts and isolated ventricular myocytes had no observed effects on cardiac contractility or coronary perfusion pressure. Intracoronary administration of the sEH inhibitor AUDA produced a dose-related reduction in infarct size in dogs. AUDA also enhanced the cardioprotective actions of 14, 15-EET when coadministered. More recently, cardioprotective effects of

a compound with dual EET agonist and sEH inhibitor properties, UA-8 significantly improved left ventricular developed pressure (LVDP) and reduced infarct size following ischemia reperfusion. Protect brain by improving vascular function and prevent neurons from apoptosis.

Experimental studies in genetically manipulated mice have also demonstrated a cardioprotective role for EETs. Cardiac myocyte overexpression of human CYP2J2 in mice also improved cardiac function in hearts subjected to global ischemia followed by reperfusion. Postischemic electrocardiogram abnormalities were also prevented by cardiomyocyte-specific overexpression of CYP2J2. Administration of epoxygenase enzyme inhibitors or the EET antagonist 14, 15-EEZE prevented the beneficial cardiac effects observed in the *Ephx2* −/− and CYP2J2 transgenic mice. These studies clearly demonstrate that pharmacological or genetic manipulation of EETs can protect the heart from ischemia reperfusion injury.

Additional mechanistic studies have determined that a key component to the cardiac protection afforded by EETs is K_{ATP} channel activation. Experimental studies in dogs and mice have demonstrated that the cardioprotective actions of EETs can be abolished by the nonselective K_{ATP} channel antagonist glibenclamide.

EETS ANALOGE AND AGONIST

- Analog-NUDSA
- Dialate afferent arteriole to similar extent as 11,12-EET and lower blood pressure in angiotensin -dependent hypertension
- Increased renal Cyp2c44 expression
- Dual EET agonist and SEH inhibitor-UA-8
- Significantly improve left ventricular developed pressure (LVDP) and reduce infarct size following ischemia reperfusion

CONCLUSION

EETs have many actions that contribute importantly to cardiac and vascular physiology to maintain cardiovascular homeostasis. The development of novel pharmacological and molecular biological tools such as EET agonists and antagonists will be necessary to properly define the cardiovascular actions of EETs. Angiogenic action of EETs has resulted in investigation of this pathway in tumor and cancer biology.

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